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Pharmacological studies of human erectile tissue: characteristics of spontaneous contractions and alterations in α-adrenoceptor responsiveness with age and disease in isolated tissues

[1]George J. Christ, *Saul Maayani, Mira Valcic & Arnold Melman

Department of Urology, Montefiore Medical Center of the Albert Einstein College of Medicine, Bronx, NY 10467, and *Departments of Anesthesiology and Pharmacology, Mount Sinai School of Medicine, CUNY, NY 10029, U.S.A.

1 The pathophysiology of impotence related to vascular smooth muscle dysfunction in the male corpus cavernosum was studied on human isolated erectile tissue (HET). Studies were conducted on 140 sections of HET obtained from 38 male patients undergoing surgery for implantation of penile prostheses to correct underlying erectile dysfunction.

2 Spontaneous myotonic oscillations were characteristic of greater than 90% of all HET preparations at 37°C. These spontaneous oscillations were markedly attenuated by indomethacin, BW755C, nifedipine, removal of extracellular Ca^{2+}, or lower temperatures ($\leqslant 32°C$), but were not sensitive to inhibition by atropine, phentolamine or tetrodotoxin. Our data suggest that the oscillations may, at least in part, result from the generation and/or release of a stable cyclo-oxygenase product and a consequent increase in transmembrane Ca^{2+} influx.

3 The phenylephrine-induced contractions in HET may be reliably assayed up to 24 h after surgical removal, without significant alterations in the EC_{50}, maximum response (E_{max}) or slope index of the steady-state concentration-response curve to phenylephrine.

4 The competitive and surmountable nature of the antagonism of phenylephrine-induced contractions by prazosin and yohimbine allowed calculation of antagonist dissociation constants. The calculated pK_b values for prazosin and yohimbine, respectively, were 9.47 ± 0.49 and 5.54 ± 0.22. The rank order of agonist potency in HET was: noradrenaline = phenylephrine $\gg$ clonidine. These data indicate the presence of a population of membrane receptors that are predominantly of the α_1-adrenoceptor subtype.

5 The entire patient population was stratified on a decennial basis into five age groups, and each age group was subsequently subdivided into diabetic and nondiabetic diagnostic categories. With respect to the steady-state phenylephrine concentration-response curves, a Winer two-factor analysis of variance revealed a significant effect of age on the calculated pEC_{50} value, as well as a significant age-diagnosis interaction. A *post hoc* statistical analysis for unpaired samples yielded significant differences between pEC_{50} values for diabetic and nondiabetic patients in age groups 41–50 and 61–70 years. In addition, a Winer two-factor analysis of variance also detected a significant effect of age on the calculated E_{max} value.

6 In conclusion, our studies demonstrate that spontaneous contractions in HET are likely to be mediated by the generation and release of a stable cyclo-oxygenase product. Furthermore, the results of both agonist and antagonist studies are consistent with the presence of a homogeneous α_1-adrenoceptor population. Lastly, the responsiveness of isolated HET to phenylephrine was shown to be altered by both age and disease.

Introduction

Alterations in tissue responsiveness to endogenous substances may play a role in the aetiology of a variety of vascular disease states. For example, such alterations are among the contributing factors to the vascular complications of diabetes mellitus (Brody & Dixon; 1964; Fortes *et al.*, 1983; Factor *et al.*, 1984; White & Carrier, 1986) and perhaps essential hypertension, coronary vasospasm, Raynaud's syndrome (van Zwieten, 1987; Vanhoutte, 1987; Hollenberg, 1987) and male impotentia as well (see below). However, despite recently demonstrated interspecies differences in vascular responsiveness to endogenous substances (Malomvolgyi *et al.*, 1988), relatively little work has focused on the study of vascular pathophysiology utilizing human isolated tissue preparations (Docherty, 1987; Godfraind *et al.*, 1988).

The human erectile tissue (HET) preparation has been used by several laboratories to study the pharmacology of human vascular smooth muscle (Adaikan & Karim, 1981; Fovaeus *et al.*, 1987; Heaton, 1989; Saenz de Tejada *et al.*, 1989a,b; Christ *et al.*, 1989). HET is a readily available smooth muscle preparation obtained from the vascular sinusoids of the male corpus cavernosum. The therapeutic success of intracorporal pharmacotherapy in the treatment of erectile dysfunction has highlighted the central role of corporal smooth muscle in the erectile process and has revealed that relaxation of this tissue is impaired in a large proportion of impotent men (Melman *et al.*, 1981; Juenemann *et al.*, 1986; Lue & Tanagho, 1987; Christ *et al.*, 1989; Saenz de Tejeda *et al.*, 1989a). Thus, isolated HET is a natural preparation for the study of potential age- and disease-related alterations in erectile function, and perhaps other human vascular smooth muscle disorders as well.

The current preponderance of evidence, both *in vitro* and *in vivo*, indicates that maintenance of the flaccid penile state is, at least in part, related to release of noradrenaline from sympathetic nerves and contraction of corporal smooth muscle subsequent to activation of a homogeneous postsynaptic α-adrenoceptor population (Adaikan & Karim, 1981; Hedlund & Andersson, 1985a; Saenz de Tejada *et al.*, 1989a,b). Although binding studies in membrane preparations of HET also suggest the presence of a single population of α-adrenoceptors (Levin & Wein, 1980), a conclusive pharmacological classification of the receptor that mediates

[1] Author for correspondence.

phenylephrine- and noradrenaline-induced contractions at the intact tissue level, as either the α_1- or α_2-adrenoceptor subtype, is still lacking. Additionally, there are no rigorous descriptions of the characteristics of the spontaneous contractile activity commonly observed in HET *in vitro*. This paper addresses these issues, details the conditions under which isolated HET may be reliably used to study some aspects of the pharmacology of human vascular smooth muscle and demonstrates that the characteristics of phenylephrine-induced steady-state contractions in HET are both age- and pathology-dependent.

Methods

Tissue preparation

Four to eight sections of human corpus cavernosal tissue were obtained from each of 38 patients undergoing surgery for implantation of penile prostheses. The patients age ranged from 31 to 84 years, and this population consisted of three broad diagnostic groups; (1) psychogenic impotence, (2) impotence due to organic dysfunction (e.g. diabetes, Peyronie's disease, vasculogenic), and (3) impotence secondary to pharmacotherapy (e.g., β-adrenoceptor blockade).

Sections of HET approximately 2 × 2 × 6 mm in size, were suspended between two small fish hooks in 20 ml organ chambers containing Krebs-Henseleit buffer of the following composition (mM): NaCl 110, KCl 4.80, $CaCl_2$ 2.5, $MgSO_4$ 1.20, KH_2PO_4 1.20, $NaHCO_3$ 25.0 and dextrose 11.0, in glass distilled water. Organ chambers were maintained at 37 ± 1°C and were continuously aerated with 95% O_2 and 5% CO_2 to maintain pH = 7.4 ± 0.1. The sections of HET were initially suspended at 2–3 g basal tension, washed periodically with fresh buffer, and allowed to stabilize at 37°C over a period of 90–120 min. When a stable resting tension was attained, the tissues were set to 2 g basal tension.

Tissues were primed twice (at 37°C) by the addition of 10 μM phenylephrine to the organ bath. Contractions were measured isometrically with a Grass Force Displacement Transducer (Model FT-03), and recorded on a Grass Polygraph (Model 7D). After a steady-state contraction was achieved, the drug was removed from the tissue by replacing the contents of the bath at least 3 times with fresh buffer.

Construction of concentration-response curves (CRCs) and receptor classification studies

For these studies, the organ bath temperature was lowered to 32°C and concentration-response curves to phenylephrine, noradrenaline or both were constructed by the cumulative addition of drug at half log unit increments. In the α-adrenoceptor classification studies, a control concentration-response curve to phenylephrine was first obtained, and then the tissues were washed with fresh buffer several times and allowed to relax to baseline tension over the next 60–90 min. When a stable baseline tension was reached, either prazosin (0.5 nM) or yohimbine (3–100 μM) was added for 30 min before construction of a second concentration-response curve on the same tissue.

Stratification of the patient population

For the purposes of statistical analysis, the patient population was stratified by decade into 5 age groups (see Results), with each age group subsequently subdivided into two distinct diagnostic groups: (1) those patients whose impotence was associated with diabetes mellitus (both insulin-dependent and non-insulin-dependent diabetics were grouped together) and (2) those patients with impotence associated with organic manifestations other than diabetes (see above) or who were diagnosed as having psychogenic impotence.

Data analysis

The magnitude of the spontaneous oscillations was calculated from the index recently proposed by Katusic *et al.*, (1988). Briefly, rhythmic activity was quantified by eye, as the product of the mean contractile amplitude and the frequency during a 10 min period.

Concentration-response curve data were analyzed on an IBM compatible computer using the RS/1 software package (BBN; Cambridge, MA). Briefly, concentration-response curve data were fitted to a simple logistic equation of the form:

$$E = E_{max}/\{1 + (EC_{50}/[D]^{n_H}\} \quad (1)$$

where E is the observed effect in g of tension, [D] is the molar concentration of drug, E_{max} is the calculated maximal effect, EC_{50} is the concentration of agonist necessary for one half E_{max}, and n_H is the slope factor. EC_{50} values are expressed as the negative logarithm of the geometric mean (pEC_{50}) ± s.e.mean, while all other logistic parameters are expressed as the arithmetic mean ± s.e.mean.

Since Chi-Square analysis revealed that the slope of the Schild plot (Arunlakshana & Schild, 1959) for yohimbine antagonism of the phenylephrine-induced contraction in HET was not significantly different from unity, and that prazosin antagonism of the phenylephrine-induced contraction was also consistent with competitive and surmountable antagonism, antagonist dissociation constants were determined from Equation 2:

$$pK_b = \frac{\log[B]}{DR - 1} \quad (2)$$

where B is the molar concentration of antagonist, DR (dose ratio) is the ratio of the EC_{50} doses before and after addition of antagonist to the organ bath and pK_b is the antagonist dissociation constant, expressed as the negative logarithm of the geometric mean ± s.e.mean.

Statistical analysis

All statistical calculations were performed with Apple Macintosh-based Statview II software. A Winer two-factor analysis of variance for balanced samples was used to assess the effects of age and disease on the logistic parameters E_{max} and pEC_{50}. When a significant effect of age was detected, a *post hoc* Scheffe test was used for all paired comparisons among age groups. When a significant age-diabetes interaction was detected, a *post hoc* Scheffe test was also used for all paired comparisons among age groups within each diagnostic category and Student's *t* test for unrelated samples was used for comparison between diagnostic categories within each age group.

Phenylephrine, noradrenaline, prazosin, yohimbine, phentolamine, atropine, propranolol, ethyleneglycol-*bis*-N,N,N,N-tetraacetate (EGTA), tetrodotoxin, indomethacin, hydrocortisone and clonidine were obtained from Sigma Chemical Company (St Louis, MO, U.S.A.). Nifedipine and Bay K 8644(1,4-dihydro-2,6-dimethyl-5-nitro-4-[2-(trifluoromethyl)-phenyl]-3-pyridinecarboxylic acid methyl ester) were obtained from Research Biochemicals Incorporated (RBI, Natick MA, U.S.A.), cocaine was obtained from the Montefiore Hospital Pharmacy, and BW755C (3-amino-1-[*m*-(trifluoromethyl)-phenyl]-2-pyrazoline) was the generous gift of Dr Michal Schwartzman; other compounds were of analytical grade.

Results

Characteristics of the spontaneous myotonic oscillations

During the initial tissue equilibration period at 37°C (90–120 min, see Methods), spontaneous myotonic oscillations

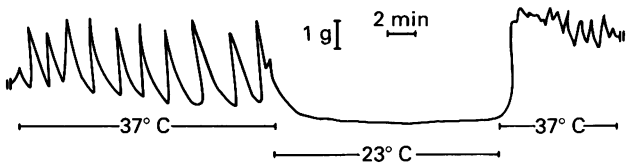

Figure 1 Representative tracing of an original polygraph recording, illustrating the temperature-sensitivity of the spontaneous myotonic oscillations in human isolated erectile tissue. Note changes in frequency and amplitude of the oscillations.

occurred in greater than 90% of all erectile tissue preparations from the same patient (Figure 1). The frequency and amplitude of the spontaneous contractions exhibited substantial variation among tissue sections taken from different patients as well as among individual tissue sections taken from the same patient. For example, attempts to quantitate the spontaneous oscillations using an index recently proposed by Katusic *et al.* (1988), revealed that the spontaneous activity varied over time by as much as 40% in the same HET preparation, while exhibiting greater than 10 fold variation among 8 different HET preparations from the same patient. Therefore, to permit pharmacological studies in this preparation, it was necessary to characterize some aspects of this oscillatory behaviour.

The observed spontaneous oscillatory contractions can be distinguished from sustained phenylephrine-induced contractions based on variety of criteria, as listed in Table 1. For example, the appearance of spontaneous oscillatory activity was inversely related to the ambient temperature; that is, oscillations were much less likely to occur at 32°C (not shown) than 37°C and were completely eliminated at 23°C (Figure 1). Figure 2a illustrates that chelation of extracellular Ca^{2+} by cumulative addition of EGTA caused a gradual cessation of spontaneous activity. Similarly, replacement of the Krebs buffer with Ca^{2+}-free/2 mM EGTA buffer also terminated spontaneous activity (Figure 2b). Addition of 0.1 μM nifedipine either prevented the appearance of the spontaneous myotonic oscillations or abolished those that were present (Figure 3), while the addition of 0.1 μM Bay K 8644 accelerated the appearance of oscillatory contractions in previously quiescent tissues, or amplified pre-existing oscillatory activity (Figure 3). The effects of nifedipine and Bay K 8644 were mutually antagonistic, regardless of their order of addition.

Addition of 10 μM indomethacin significantly attenuated the spontaneous activity in tissues taken from 7 out of 8 patients, as follows: it abolished the oscillations in 4 subjects, and decreased the amplitude and/or frequency of the spontaneous contractions in the HET preparations of 3 other patients, with no apparent effect on only 1 subject. BW755C (10–50 μM) caused a complete cessation of the spontaneous contractions in all tissues taken from 3 patients. Atropine (1 μM, 1 subject), phentolamine (1 μM, 1 subject), and tetrodotoxin (5 μM, 4

Table 1 Some discriminators of human erectile tissue tone generation

	Steady-state contraction phenylephrine (10 μM)	*Spontaneous oscillations*
Extracellular Ca^{2+} dependence	Yes	Yes
Nifedipine sensitivity (10 nM)	No	Yes
Bay K 8644 sensitivity (10 nM)	No	Yes
Temperature sensitivity	No	Yes
Prazosin or phentolamine sensitivity (1 μM)	Yes	No
Indomethacin sensitivity (10 μM)	No	Yes

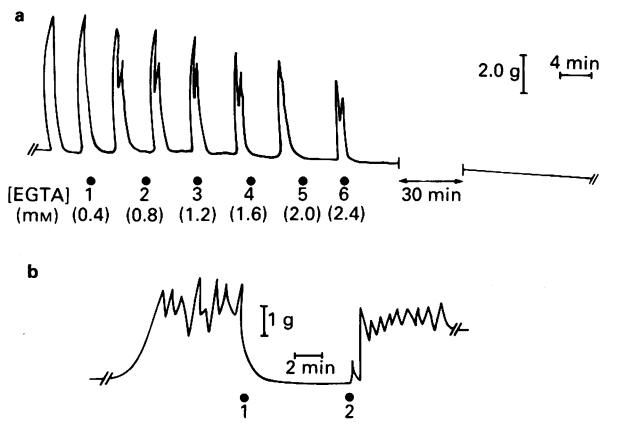

Figure 2 Dependence of spontaneous myotonic oscillations on extracellular $[Ca^{2+}]$. Illustrated are representative tracings of original polygraph recordings from two different human isolated erectile tissue preparations maintained at 37°C. Similar results were obtained in tissues from several other patients (not shown). Points 1–6 in (a) represent the cumulative addition of EGTA in 0.4 mM aliquots; note that the oscillations were restored upon re-addition of extracellular Ca^{2+} (not shown). In (b), (1) = Ca^{2+}-free/EGTA buffer; (2) = 2.5 mM Ca^{2+}. Also, note the difference in scale between (a) and (b). All concentrations refer to the final concentration in the organ bath.

subjects), had no effect on spontaneous activity. Furthermore, on a number of occasions we demonstrated that one can immediately induce oscillations in a quiescent tissue by exposing it to the buffer removed from the organ bath of an oscillating tissue.

Concentration-response curves to α_1-adrenoceptor agonists and assessment of response stability in the isolated HET preparation

Addition of phenylephrine at concentrations lower than 1 μM frequently induced oscillatory contractions at 37°C, thus complicating analysis of the concentration-response curve at concentrations below the EC_{50}. Addition of higher concentrations of phenylephrine elicited sustained tonic contractions. By lowering the bath temperature to 32°C we were able to obtain complete concentration-response curves to phenylephrine that were generally free from spontaneous activity (Figure 4).

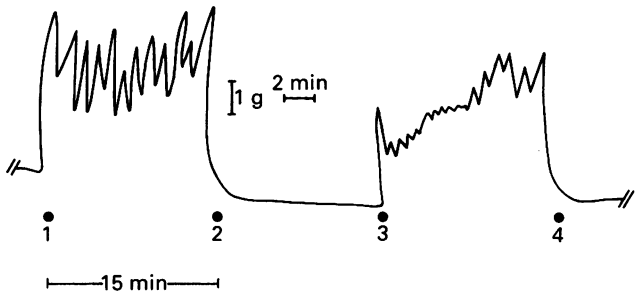

Figure 3 The effects of the dihydropyridines nifedipine and Bay K 8644 on spontaneous activity in human isolated erectile tissue (HET) and their mutual reversibility. Shown is a representative tracing of an original polygraph recording of an HET preparation maintained as 37°C, prior to the appearance of oscillatory activity. All concentrations represent the final free concentration in the organ bath. At point (1) addition of Bay K 8644 (0.1 μM) to a previously quiescent tissue elicited oscillatory activity; (2) = Ca^{2+}-free EGTA buffer which abolished oscillatory activity; (3) owing to the high lipophilicity of the dihydropyridines, they are very difficult to wash out of the tissue, thus re-addition of Ca^{2+} (2.5 mM) to the organ bath caused immediate oscillatory activity due to the presence of a residual amount of Bay K 8644; (4), the addition of nifedipine (0.1 μM) reversed the effects of Bay K 8644, and abolished spontaneous activity. Note change in time scale.

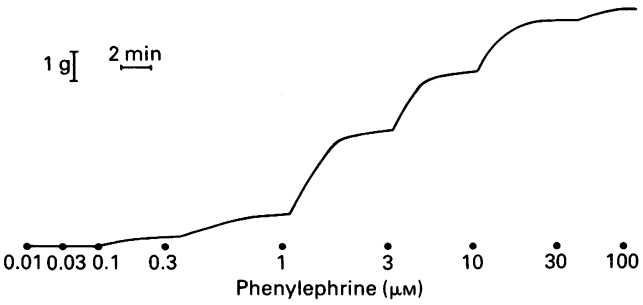

Figure 4 Representative tracing of the graded contractile response of human isolated erectile tissue to phenylephrine at 32°C. Note the absence of oscillations and the stability of the phenylephrine-induced contractile response.

By this method, concentration-response curves to phenylephrine were constructed on all 20 tissues from 5 patients at both 4 h and 24 h after dissection. A paired statistical analysis of the E_{max}, EC_{50} and slope factor values at 4 and 24 h revealed no significant time-dependent alterations of these parameters (Table 2). In addition, the maximal response to 10 μM phenylephrine was stable for up to 30 min without decay at both time intervals. However, despite the similarity in tissue size (see Methods), the calculated E_{max} values varied substantially among tissue sections taken from the same patient; 3 fold differences in maximal contraction were not uncommon.

Classification of the α-adrenoceptor subtype mediating contraction of HET

Antagonist studies Prazosin (0.5 nM) and yohimbine (3–100 μM) both produced rightward, parallel and surmountable shifts of the phenylephrine concentration-response curve that were consistent with simple competitive binding (Figure 5); while the pK_b value for yohimbine was calculated from the Schild equation (Arunlakshana & Schild, 1959), the pK_b value of prazosin was calculated using Equation 2 (see Methods). The mean pK_b values were 5.54 ± 0.22 (2.88 μM, $n = 8$ tissues from 3 patients) for yohimbine, and 9.47 ± 0.49 (0.34 nM, $n = 5$ tissues from 4 patients) for prazosin. These pK_b values are similar to those reported for antagonist inhibition of α-adrenoceptor responses in other isolated tissue preparations (Timmermans & Van Zwieten, 1982; Minneman & Abel, 1987; Bevan *et al.*, 1989) and reflect the predominance of the α_1-adrenoceptor subtype in HET.

Agonist studies Further receptor classification studies were performed with three agonists; phenylephrine, noradrenaline, and clonidine. Cumulative addition of phenylephrine (Figure 4) or noradrenaline (in the presence of metabolic inhibitors and tissue uptake blockers, see Methods) elicited graded contractile responses in HET. Computer fits of Equation 1 to observed concentration-response curve data yielded mean E_{max}, pEC_{50} and slope factor values of 5.36 ± 0.17, 5.94 ± 0.03 (1.15 μM) and 1.00 ± 0.02, respectively, for phenylephrine (158 tissue preparations from 39 patients), and 4.40 ± 0.50, 6.10 ± 0.08 (0.79 μM) and 0.97 ± 0.05 respectively for noradrenaline (14 tissue preparations from 5 patients). Paired statistical analysis of the concentration-response curve parameters for phenylephrine and noradrenaline on the same

Table 2 Time-dependent effects on phenylephrine-induced contraction

	E_{max} (g)	*Slope* pEC_{50}	(n_H)
Day 1 (4 h)	5.3 ± 0.47	6.06 ± 0.13	0.95 ± 0.05
Day 2 (24 h)	4.9 ± 0.56	5.86 ± 0.09	0.99 ± 0.07

The pEC_{50} values are given as the geometric mean ± s.e.mean, while all other values represent the arithmetic mean ± s.e.mean. The sample size (n) for phenylephrine is 20 tissue preparations from 5 patients. Paired analysis revealed no significant differences between Day 1 and Day 2 for any of the parameters derived from computer fits of the logistic equation to phenylephrine concentration-response curve data.

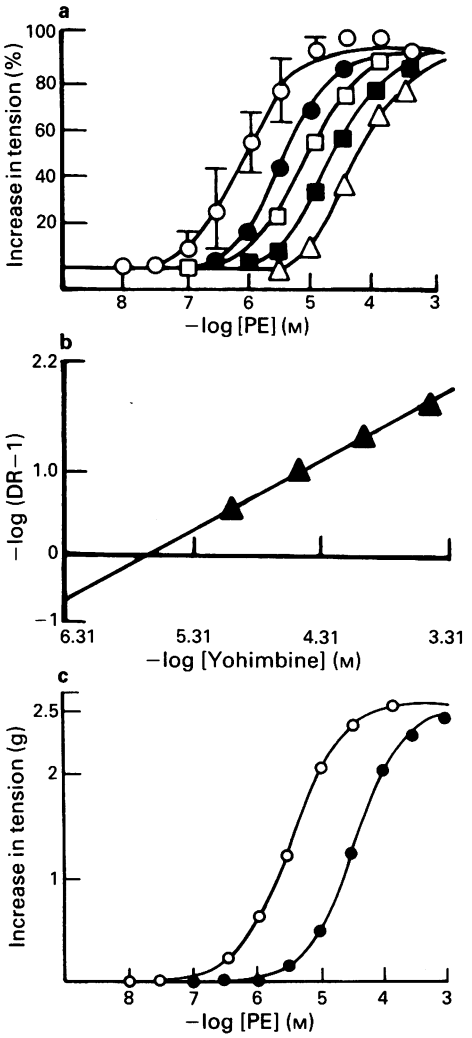

Figure 5 Characterization of the α-adrenoceptor subtype which mediates phenylephrine-induced contraction in human isolated erectile tissue. (a) Concentration-response curves to phenylephrine were normalized as a percentage of the maximal observed response to phenylephrine in each of four different tissues from the same patient. The control curve (○) represents the mean value (with s.e.mean shown by vertical bars) for all four tissues. Each tissue was then preincubated with a single concentration of yohimbine; 3 (●), 10 (□), 30 (■), or 100 (△) μM respectively, before repeating the concentration-response curve to phenylephrine. All concentration-response curves were computer-fitted to the logistic equation. (b) Schild plot of the concentration-response curve data from (a). The slope of the Schild plot was not different from unity, therefore, the slope was constrained to unity, and the K_b value was calculated from the Schild equation ($K_b = 1.99$ μM; $pK_b = 5.70$). (c) Representative computer-fitted curves to observed concentration-response curve data in the absence (○) and presence (●) of prazosin (0.5 nM). The prazosin K_b value was calculated using Equation 2 (see Methods) ($K_b = 50$ pM; $pK_b = 10.3$). Notice the complete surmountability and the parallel rightward shift in the phenylephrine concentration-response curve produced by both yohimbine and prazosin. All concentration-response curve data were collected at 32°C.

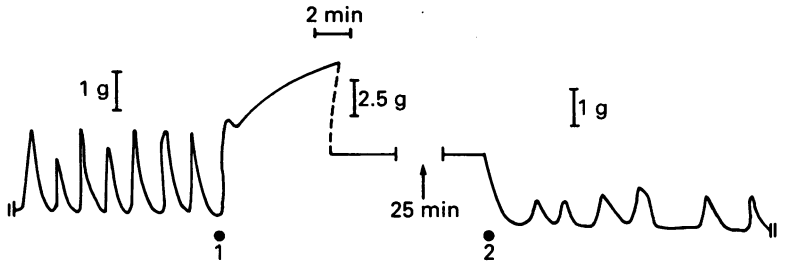

Figure 6 Reversible 'masking' of spontaneous contractions of human isolated erectile tissue (HET) by phenylephrine. The tonic contraction to 10 μM phenylephrine (1) reversibly 'masks' characteristic spontaneous activity. Shown is a representative tracing of an original polygraph recording taken from an HET preparation maintained at 37°C. Note the change in scale after the addition of phenylephrine and the change in the characteristics of the spontaneous activity upon removal of phenylephrine from the organ bath (2).

tissues revealed no significant differences. Furthermore, the contractile response to clonidine was variable and much smaller than that to phenylephrine. In addition, there was no detectable response to clonidine in the presence of 1 μM prazosin.

Effect of age and diagnostic category on the phenylephrine-induced steady-state response

The effects of the subject age and diagnostic category on the phenylephrine concentration-response curve parameters described above were evaluated. For this analysis, the entire patient population was stratified on a decennial basis, and each age group was subsequently subdivided into diabetic and nondiabetic diagnostic categories, respectively (see Methods). The value of the slope parameter (n_H) was not significantly different from unity (as determined by Chi-Square analysis) and thus was not included in this analysis. However, a Winer two-factor analysis of variance revealed a significant effect of age on the calculated pEC_{50} value ($P < 0.01$), as well as a significant age-diagnosis interaction ($P < 0.03$), but failed to detect any effect of diagnosis alone ($P = 0.43$). A *post hoc* Scheffe test revealed significant differences in the pEC_{50} values among the 5 age groups (Figure 7a). Simple linear regression analysis of pEC_{50} versus age for the diabetic tissues illustrates that the pEC_{50} value increases significantly with increasing age (correlation coefficient, $r = 0.82$, Figure 7b). There was no significant correlation between pEC_{50} and age for the nondiabetic tissues ($r = 0.06$). *Post hoc* comparisons of pEC_{50} values between the diabetic and nondiabetic categories within each age group revealed significant differences between the 2 diagnostic categories in age groups 41–50 and 61–70 (Table 3). In addition, paired comparisons among the 5 age groups within each diagnostic category revealed significant differences in pEC_{50} values for both diabetic and nondiabetic tissues (Table 3).

With respect to E_{max}, a Winer two-factor analysis of variance revealed a significant effect of age ($P < 0.01$), but no effect of diagnosis ($P = 0.86$), and no age-diagnosis interaction ($P < 0.06$). A *post hoc* Scheffe test yielded significant differences among the 5 age groups in the calculated E_{max} values (Figure 8b). The linear regression analysis shown in Figure 8b demonstrates that increases in E_{max} were significantly correlated with increasing age ($r = 0.85$).

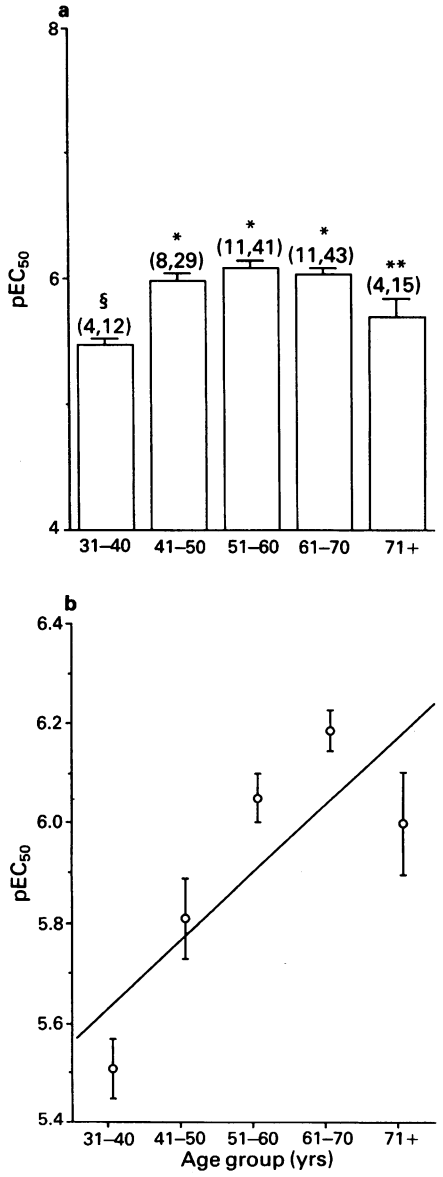

Figure 7 Effect of age and diagnosis on the pEC_{50} value. (a) Depicts the mean calculated pEC_{50} (negative logarithm of the geometric mean) values for each age group: *denotes significant difference from age group 31–40, $P < 0.05$; **denotes significant difference from age group 61–70, $P < 0.05$. (b) Depicts a simple linear regression of the mean pEC_{50} value versus age for the diabetic tissues and shows that the pEC_{50} is strongly positively correlated with age (correlation coefficient, $r = 0.82$). All values represent the mean with s.e.mean shown by vertical bars. In (a), §number to the left of comma denotes the number of subjects, while the number to the right of the comma denotes the total number of tissues; the number of subjects and tissues is the same for (a) and (b).

Discussion

One of the most noteworthy features of the isolated HET preparation is the characteristic myotonic automaticity observed at 37°C (Figure 1, Saenz de Tejada *et al.*, 1989b). The spontaneous contractions described here exhibited considerable variation with respect to both their amplitude and frequency (between patients, as well as among tissue sections taken from the same patient), and thus eluded attempts at a

Table 3 Effects of age and disease on the sensitivity of phenylephrine-induced contractions in human isolated erectile tissue

	Age group (years)				
pEC_{50}	31–40	41–50	51–60	61–70	71+
Diabetic	5.50 ± 0.06 (1,4)[a]	5.80[e] ± 0.08 (3,10)	6.04[c] ± 0.06 (6,24)	6.18[c] ± 0.06 (3,14)	5.99 ± 0.10 (1,4)
Nondiabetic	5.46 ± 0.09 (2,8)	6.07[b,c] ± 0.06 (5,19)	6.13[c,d] ± 0.11 (5,17)	5.96[b] ± 0.07 (8,29)	5.59 ± 0.18 (3,11)

[a] The number in parentheses to the left of the comma denotes the number of patients in each category, while the number to the right of the comma denotes the total number of tissues. [b] Denotes statistically significant difference from corresponding diabetic value within the same age group. [c] Denotes statistically significant difference from age group 31–40 within the same diagnostic category. [d] Denotes statistically significant difference from age group 71+ for the nondiabetic category. [e] Denotes statistically significant difference from age group 61–70 for the diabetic category. Statistical significance was determined as $P < 0.05$. pEC_{50} represents the negative logarithm of the geometric mean ± s.e.mean; where the s.e.mean is based on the total number of tissues.

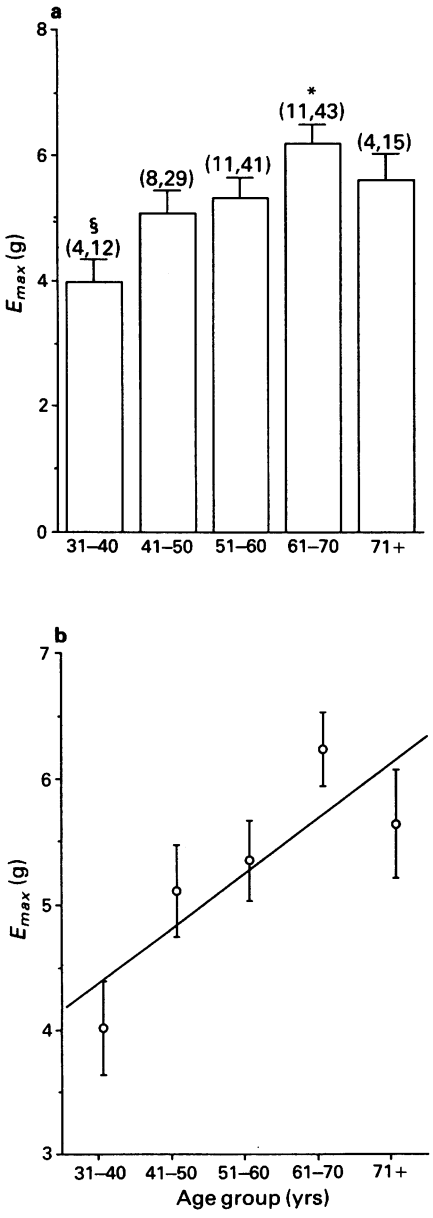

Figure 8 Effect of age on the phenylephrine E_{max}. (a) Depicts the mean E_{max} values for each age group: *denotes significant difference from age group 31–40, $P < 0.05$. (*b*) Depicts a simple linear regression of the mean E_{max} value versus age and shows that E_{max} is strongly positively correlated with age (correlation coefficient, $r = 0.85$). All values represent the mean with s.e.mean shown by vertical bars. In (a), §number to the left of comma denotes the number of subjects while the number to the right of the comma denotes the total number of tissues; the number of subjects and tissues is the same for (a) and (b).

quantitative description (see Results). Despite these limitations, our studies have revealed many interesting qualitative features of the spontaneous contractions. The fact that one can immediately induce oscillations in a quiescent tissue by exposing it to the buffer removed from the organ bath of an oscillating tissue strongly suggests that the oscillations are, at least in part, related to the generation and release of a stable endogenous substance.

The failure of atropine and phentolamine to inhibit spontaneous contractions demonstrates that the source of these contractions is not a rhythmic release of either endogenous acetylcholine or noradrenaline. In contrast, the inhibition of the spontaneous oscillations by either indomethacin or BW755C indicates that the oscillations may be mediated by products of the cyclo-oxygenase pathway, as we have previously suggested (Melman *et al.*, 1986) and has been shown in human isolated saphenous vein (Schoeffter & Godfraind, 1989). In fact, prostaglandin synthesis has been demonstrated in HET *in vitro* (Roy *et al.*, 1984), and addition of prostaglandin $F_{2\alpha}$ to HET actually induces rhythmic contractile activity (unpublished observations; Roy *et al.*, 1984; Hedlund & Andersson, 1985b). The properties of the spontaneous myotonic oscillations in HET described here are similar to those reported for phentolamine-induced oscillations in the guinea-pig bladder detrusor muscle (Satake *et al.*, 1984), as well as KCl-induced oscillations in the canine basilar artery (Vanhoutte, 1988). In the basilar artery, it was suggested that the rhythmic pattern of contraction was due to activation of endothelial cyclo-oxygenase with the subsequent release of vasoconstrictor prostaglandins and of prostacyclin; the latter of which exerts an inhibitory feedback on the activated cyclo-oxygenase, thus ensuring rhythmic contractions. Since electron microscopy has demonstrated the presence of endothelial cells in the HET preparation (unpublished observations), and other laboratories have shown this endothelium to be functionally active (Saenz de Tejada *et al.*, 1989a), a similar mechanism to that suggested for canine basilar artery may be responsible for generation of spontaneous activity in HET.

Previous studies concerning the classification of the α-adrenoceptor subtype mediating contraction in HET have relied primarily on the rank order of agonist potency, displacement of agonist concentration-response curves by a single concentration of antagonist (without calculation of antagonist affinity) and the relaxation of precontracted HET by selective α-adrenoceptor antagonists (Adaikan & Karim, 1981; Hedlund & Andersson, 1985a; Saenz de Tejada *et al.*, 1989b). Our studies include pK_b values calculated from antagonist studies under well established equilibrium conditions (Figure 5, see Results) and demonstrate that the phenylephrine- and noradrenaline-induced contractions in isolated HET are mediated by activation of predominantly α_1- and not α_2-adrenoceptors. In addition, we demonstrated a rank order of agonist potency consistent with the presence of a predominantly α_1-adrenoceptor population; i.e., noradrenaline = phenylephrine $\gg$ clonidine.

Pharmacological studies on human isolated vascular smooth muscle tissues represent an initial step toward understanding mechanisms of contraction and relaxation *in vivo* and, ultimately, toward characterization of alterations in vascular smooth muscle function related to age and pathology. At present, we are using the steady-state logistic parameters as an index for detecting fundamental alterations in the pharmacology of vascular smooth muscle with age or disease. This is because a significant difference in any one of the logistic parameters, whether among age groups or between diabetic and nondiabetic patients within the same age group, reflects a fundamental difference in the phenylephrine concentration-response relationship.

Our data demonstrate that the pEC_{50} was subject to significant variation with age (Figure 7a) and that, for the diabetic tissues but not the nondiabetic tissues, pEC_{50} was positively correlated with age (Figure 7b). These age- and pathology-dependent characteristics result in significant differences in the pEC_{50} between diabetic and nondiabetic tissues in certain age groups (Table 3). In addition, we demonstrated that the maximal phenylephrine-induced contraction in HET (E_{max}) is also positively correlated with age. Despite this strong correlation, a statistically significant difference in the mean E_{max} values among the 5 age groups was found only between age groups 31–40 and 61–70.

In conclusion, our studies demonstrate that the spontaneous contractile activity observed in HET is likely to be mediated by the generation and release of a stable cyclooxygenase product. Furthermore, the results of steady-state studies with both agonists and antagonists are consistent with the presence of a homogeneous α_1-adrenoceptor population. Lastly, the responsiveness of isolated HET to phenylephrine was shown to be altered by both age and disease.

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